REMARKS/ARGUMENTS

Applicants would like to thank Examiners Long Le and Jacob Cheu for the courtesies extended during the telephonic interview with Applicants' representatives, Ferris Lander and Kathryn Bex, conducted on October 12, 2005.

Applicants note that the instant Office action contains numerous inconsistencies. Applicants telephoned the Examiner on November 09, 2005 to request clarification by way of a supplemental Office action. It was agreed that rather than issuing a supplemental action the various inconsistences will be addressed and corrected via the Response by Applicants herein. That is, all recitations of "claim 1" should be --claims 1, 44-46-- (as claims 1, 44-46 are currently under examination); "SEQ ID NOS.1-3" should be --SEQ ID NOS. 1-4-- (as these peptide sequences are currently claimed); and "insulin resistance patients" should be --Alzheimer's patients--. Additionally, "band #9" should be --band 1-- (see penultimate paragraph, page 3 of the instant Office Action) and "...mass spectrum of the peptides in Figure 2 and 4..." should be --mass spectrum of the peptides in Figure 2-- (as there is no Figure 3 or 4 in the present application), see last paragraph, page 3 of the instant Office action.

It is hereby requested that should Applicants have misunderstood or in any way misinterpreted the outstanding Office

action, the Examiner will issue a new non-final Office action to correct these inconsistencies.

In response to the Office Action of November 01, 2005, Applicants request re-examination and reconsideration of this application for patent pursuant to 35 U.S.C. 132.

Claim Status/Support for Amendments

Claims 1, 39-46 are currently pending. Claims 1, 39, 42 and 44 have been amended herein. Claims 2-38 were cancelled in a previous response. Claims 39-43 were withdrawn from consideration. Claims 1, 44-46 are currently under examination.

In view of the present amendments agreed to during the aforementioned telephonic interview of October 12, 2005, examined claims 1, 44-46 (Group I) are now believed to be in condition for allowance. Claims 39-43 are drawn to the non-elected invention. Applicants respectfully request rejoinder of the remaining claims (39-43), in accordance with the decision in *In re Ochiai*, since the remaining claims (39-43) are limited to the use of the biopolymer markers of claim 1 (the examined claim of the elected Group I invention). If the biopolymer marker peptide of claim 1 is found to be novel, methods limited to its use should also be found novel.

No new matter has been added by the amendments to the claims made herein.

In claims 1, 39 and 44, the alternative expression of the Markush format has been replaced by "or" terminology to more clearly set forth Applicants' intended scope for the biopolymer marker (see MPEP 2173.05 (h) I, II).

Claim 39 has been further amended to avoid a possible rejection under 35 USC 112, second paragraph, as suggested by the Examiner during the telephonic interview of October 12, 2005, the phase "in a manner effective to maximize analysis of" in claim 39, step (b) has been replaced with --to elucidate--. Support for this amendment can be found throughout the specification as originally filed, see, for example page 35, lines 19-22.

Claim 42 has been amended to define the acronyms for the recited mass spectrometry procedures. These acronyms are well known to those of skill in the art and are defined in various parts of the specification as originally filed, see, for example page 10, lines 2-11.

Rejection under 35 USC 112, first paragraph

Claims 1, 44-46, as filed on June 30, 2005, stand rejected under 35 USC 112, first paragraph, as allegedly failing to comply with the enablement requirement. The Examiner asserts that the claim contains subject matter which was not described in the specification in a such a way as to enable one skilled in the art

to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The Examiner maintains the assertion that the instant invention would not enable one of ordinary skill in the art to use this invention without undue experimentation. The Examiner reiterates that there is no explanation or illustration of the significance or relationship between the peptide fragments (SEQ ID NOS:1-4) and Alzheimer's disease as seen from the view of the mass spectral profile of the peptides as in Figure 2. Figure 2 is merely a trypsin digest of fibronectin band 1. There is allegedly no indication which graph represents patients with Alzheimer's disease. There is no indication where are the SEQ ID NOS fragments or the corresponding relationship to Alzheimer's disease. Thus, the Examiner concludes there lacks a scientific nexus between the mass spectrum of the recited SEQ ID NOS:1-4 and the target disease.

Applicants respectfully disagree with the Examiner's assertions.

The "test of enablement" is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the prior art without undue experimentation (see MPEP 2164.01).

As set forth by Applicants in the previous response(filed June 30, 2005) and Declaration (and Figures) under 37 CFR 1.132 (mailed

July 10, 2003), the gel photographed in Figure 1 shows a comparison of the protein content of samples obtained from patients having a history of Alzheimer's disease with the protein content of patients not inflicted with Alzheimer's disease.

Consider, for example, band #1 which corresponds to the fibronectin protein, as disclosed on the bottom of original Figure 2 and page 46, lines 2-10 of the specification as originally filed. The identified peptide fragments of fibronectin are disclosed as SEQ ID NOS: 1-4 which correspond to the 1356, 1625, 1818 and 1629 dalton markers, respectively. Band # 1 appears strongest in all of the normal control patients, that is, lanes 5-8 (as seen from the left of Figure 1) but lighter (or not at all) in the samples of patients having Alzheimer's disease, lanes 1-4. This supports the theory of the instant invention wherein fibronectin is degraded during the disease process of Alzheimer's disease, thus, the whole protein will not appear as a distinct band on the gel, as pictured in Figure 1. In other words, band #1 was identified as "differentially expressed" between a disease state (Alzheimer's patients) and a non-disease state (control patients).

Subsequently, this band #1 was excised from the gel and subjected to mass spectrometry (SELDI MS; Figure 2). The resulting mass spectral profile (sequence) was compared to a database

containing known peptides sequences and was identified as a fragments of fibronectin.

Accordingly, the mass spectral profiles of a peptide consisting of SEQ ID NO:1, or SEQ ID NO: 2, or SEQ ID NO: 3 or SEQ ID NO: 4 can potentially identify Alzheimer's disease in the patient from which the sample was obtained.

Thus, contrary to the Examiner's assertions, the instant specification does explain and illustrate the relationship between the claimed peptide fragments in figure 1 and Alzheimer's disease.

Applicants reiterate that differential expression is a significant characteristic to consider when evaluating a peptide as a potential marker.

A peptide that is identified as differentially expressed between a "disease" state and a "normal" physiological state is more often than not recognized as a potential diagnostic marker, even if the involvement of the peptide in the pathology of the disease is unknown. One of skill in the art would be familiar with this practice since it has been known in the art since at least 1992. See attached abstract of Gunnersen et al., (Proc. of the Natl. Acad. of Sci. USA 89 (24): 11949-53 1992; reference 1) in which the detection of the protein enzyme glutamine synthetase in the cerebrospinal fluid of Alzheimer's disease patients lead to the suggestion of glutamine synthetase as a potential diagnostic

biochemical marker. Moreover, the Scott D. Patterson article (Physiological Genomics 2:59-65 2000; reference 2 in Response filed June 30, 2005) further demonstrates that it is common practice to select potential disease markers by their differential expression between a disease and non-disease state.

Thus, Applicants respectfully submit that one of skill in the art would find it acceptable to refer to any of the claimed peptides (SEQ ID NOS:1-4) as markers based upon their differential expression as seen in Figure 1.

The Examiner points to a recent article (Zhang et al., Neurobiology of Aging, Vol. 26, page 207 (2005)) in questioning enablement in the instant specification. The Examiner appears to have drawn a direct parallel between the diagnostic method reported by Zhang et al., and the methods described in the instant invention, i.e., proteomic approaches for two-dimensional gel differential electrophoresis coupled with mass spectrometry analysis. The study by Zhang et al., was aiming to identify biomarkers of common age-related neurodegenerative disease. The authors identified around 30 proteins with >20% change in concentration between older and younger individuals. According to the Examiner, Zhang et al., do not conclude that these proteins are biomarkers, rather, Zhang et al., suggest the data supplied by those proteins are a "value platform" and invite further

experimentation and confirmation (see page 214, right column, second paragraph; left column, last paragraph).

In the Response to Applicant's Applicant [sic] section in the outstanding Office action, the Examiner further asserts with respect to Zhang et al., that the discrepancy of protein expression of disease versus normal patients cannot be conclusively confirmed as a biomarker for said disease because Zhang et al., raise concerns on the relationship of the disease and the expression of the peptide fragment- "...is this a cause or consequence of disruption of the blood-brain barrier during aging process as indicated by others?" (See page 214, left column, second paragraph). In addition, Zhang et al., point to a disadvantage of this approach as the researchers were unable to discern if the differences found were due to age-related changes that were highly abundant in a single individual, focused in only a few individuals, or distributed over all participants. The Examiner states that development of mass spectrometry analysis has progressed for decades, yet in the recent article of Zhang et al., uncertainty still remains and further studies are need to confirm the validity of this approach (see page 214, right column, last paragraph). Thus, the Examiner concludes in view of the aforementioned lack of predictability in the art, undue experimentation would be required to practice the claimed methods with reasonable expectation of

success.

Applicants respectfully disagree with the Examiner's reliance on the article by Zhang et al.

As noted by the Examiner, Zhang et al., was published in 2005 (received October 2003), which is more than 3 years after the filing date of the instant invention (November 23, 2001), thus, Applicants believe the reference is not relevant to the state of the art existing at the filing date of the application and cannot be used to determine whether the instant disclosure is enabled as of the filing date. It has been established that, in general, the Examiner *should not* use post-filing date references to demonstrate the patent is non-enabling (see MPEP 2164.05(a)).

Assuming, *arguendo*, Zhang et al., is a valid reference, the mere fact that something has not been done clearly is not, in itself, a sufficient basis for rejecting all applications purporting to disclose how to do it (see MPEP 2164.02).

Applicants assert that upon closer inspection the study of Zhang et al., do not parallel the methods disclosed in the instant invention. Zhang et al., used a "shotgun" proteomic approach coupled with liquid chromatography followed by mass spectrometry to identify proteins in human CSF (see abstract; page 208, left column, last paragraph). Specifically, the method of Zhang et al. used pooled CSF samples from 22 younger and 16 older subjects to

generate two pooled samples for proteomic analysis (page 208, right column, penultimate paragraph). Zhang et al., acknowledges the problem with the use of pooled CSF samples is that they were unable to determine if the differences found were due to age-related changes in a single individual, only few individuals or distributed over all participants (page 214, last paragraph).

The claimed methodology of the present invention does not use pooled samples, rather, a sample from an individual patient is obtained and at least one biopolymer marker sequence is isolated from the sample and compared to the biopolymer marker sequence as disclosed in the present invention. Unlike Zhang et al., the presence of any of the mass spectral profiles of SEQ ID NO:1-4 of the instant invention in a sample can potentially identify Alzheimer's disease in the patient from which the sample was obtained, see for example, page 46, line 11 to page 47, line 5.

The Zhang et al. publication states the aging *markers* (30 identified proteins) need to be validated (page 211, right column 2nd full paragraph). Only two proteins (agrin and hnBNPm) were then identified individually by Western blot analysis. Zhang et al., refers throughout the publication to these proteins (agrin, hnBNPm) as "protein markers", see, for example page 211, right column, 2nd full paragraph, lines 1-6 and last line; page 214, right column, lines 2-4. Thus, contrary to the Examiner assertion, Zhang et al.,

do refer to these identified proteins as potential markers, but invites further study. Therefore, Zhang et al., actually supports and validates Applicants' study.

Furthermore, as stated in Zhang et al., the researchers used 2D gel electrophoresis/MS-base proteomic analysis in conjunction with isotope-coded affinity tags (ICAT) which bind to cysteinyl groups of all cysteine-containing proteins (at page 208, left column, third full paragraph to right column first paragraph). The Zhang et al., article warns that the ICAT technique is not without limitations, the major one being that the ICAT is limited to cysteinyl-containing proteins, thereby focusing quantification on this subset of proteins only (see page 208, sentence bridging left and right columns.) As can be seen by the Sequence Listing (filed April 23, 2002), the SEQ ID NOS:1-4 disclosed in the instant invention do not contain cysteine residues in the protein fragments.

Thus, Applicants respectfully submit that the Zhang et al., reference is not analogous to the instant invention and should not be used to control enablement.

The guidelines for a "test of enablement" indicated that if a statement of utility in the specification contains within it a connotation of how to use, and/or the art recognizes that standard modes of administration are known and contemplated, 35 USC 112, is

satisfied (see MPEP 2164.01©)). The instant application discloses a method for diagnosing Alzheimer's disease through the detection of any of the claimed biopolymer markers, SEQ ID NOS:1-4. The data presented in Figure 1 clearly show a positive correlation between the claimed biopolymer markers and Alzheimer's disease. These bipolymer markers have not previously been shown to be associated with Alzheimer's disease. When a marker is discovered to be associated with a disease state, its potential for diagnostics and/or therapeutics is immediately recognized, even if the involvement of the marker in disease pathology is unknown.

As established by the above arguments, the instant specification, contrary to the Examiner's opinion, does contain proper guidance to enable one of ordinary skill in the art to practice the claimed method for diagnosing Alzheimer's disease without undue experimentation. Thus, the Examiner's argument is not sufficient to support the enablement rejection; since the association of the claimed bipolymer marker, SEQ ID NOS:1-4, with Alzheimer's disease carries with it a connotation of use for diagnostics. Moreover, the decision in *In re Brandstadter* (179 USPQ 286) has established that the evidence provided by Applicants (to overcome an enablement rejection) need not be conclusive but merely convincing to one of skill in the art (see MPEP 2164.05).

In conclusion, Applicants respectfully submit that the instant

specification, as originally filed, provides a clear explanation of the relationship between the recited peptides (SEQ ID NOS:1-4) and Alzheimer's disease. Thus, Applicants respectfully request that this rejection under 35 USC 112, first paragraph now be withdrawn.

CONCLUSION

In light of the foregoing remarks, amendments to the specification, and amendments to the claims, it is respectfully submitted that the Examiner will now find the claims of the application allowable. Favorable reconsideration of the application is courteously requested.

Respectfully submitted,

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1: Proc Natl Acad Sci U S A. 1992 Dec 15;89(24):11949-53.

Detection of glutamine synthetase in the cerebrospinal fluid of Alzheimer diseased patients: a potential diagnostic biochemical marker.

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In this report, 8- and 2-azidoadenosine 5'-[gamma-32P]triphosphate were used to examine cerebrospinal flui (CSF) samples for the presence of an ATP binding protein unique to individuals with Alzheimer disease (AD). A 42-kDa ATP binding protein was found in the CSF of AD patients that is not observed in CSF from normal patients or other neurological controls. The photolabeling is saturated with 30 microM 2-azidoadenosine 5'-[gamma-32P]triphosphate. Photoinsertion can be totally prevented by the addition of 25 microM ATP. Photoinsertion of 2-azidoadenosine 5'-triphosphate into the protein is only weakly protected b other nucleotides such as ADP and GTP, indicating that this is a specific ATP binding protein. A total of 83 CSF samples were examined in a blind manner. The 42-kDa protein was detected in 38 of 39 AD CSF samples and in only 1 of 44 control samples. This protein was identified as glutamine synthetase [GS; glutamate-ammonia ligase; L-glutamate:ammonia ligase (ADP-forming), EC 6.3.1.2] based on similar nucleotide binding properties, comigration on two-dimensional gels, reaction with a polyclonal anti-GS antibody, and the presence of significant GS enzyme activity in AD CSF. In brain, GS plays a key role in elimination of free ammonia and also converts the neurotransmitter and excitotoxic amino acid glutamate to glutamine, which is not neurotoxic. The involvement of GS, if any, in the onset of AD is unknown. However the presence of GS in the CSF of terminal AD patients suggests that this enzyme may be a useful diagnostic marker and that further study is warranted to determine any possible role for glutamate metabolism in the pathology of AD.

PMID: 1361232 [PubMed - indexed for MEDLINE]